HIGH MAST CELL DENSITY ASSOCIATED WITH GRANULOMATOUS FORMATION IN TUBERCULOUS LYMPHADENITIS

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Abstract. Mast cells are one of the main inflammatory cells involved in the pathogenesis of tuberculosis. Previous reports revealed that mast cells participated in both acute and chronic states of infection with *Mycobacterium tuberculosis* through direct contact or indirect enhancement by releasing mediators. The authors evaluated mast cell density on tissue sections of tuberculous lymphadenitis stained with 0.1% toluidine blue from 45 cases, all of which were retrieved from the surgical pathology files of King Chulalongkorn Memorial Hospital from 1999 to 2006. A number of mast cells were correlated semiquantitatively with granulomas which were formed by aggregation of epithelioid histiocytes, multinucleated giant cells, and caseous necrosis. We found that mast cell density was significantly increased in lymph nodes with greater granuloma involvement ($p = 0.030$) and multinucleated giant cell formation ($p = 0.010$). These findings indicate a significant correlation between mast cell density and the granulomatous formation responsible for *M. tuberculosis*.

INTRODUCTION

Despite the worldwide use of vaccines and antibiotics, infection with *Mycobacterium tuberculosis* continues to be a major public heath problem in developing countries, claming 2 million lives annually (Nunn, 2001). In Thailand, the prevalence rate increased each year from 1998 to 2000 with a rate of 51.82 per 100,000 population. The death rate from all types of tuberculosis was 14.8 per 100,000 population in 2003 (Lertmaharit *et al*, 2005).

Pathogenesis of tuberculosis in human occurs predominantly in the lung. After penetrating the mucosal barrier, the inflammatory response is nonspecific, similar to the reaction against any form of bacterial invasion (Raja, 2004). Most *M. tuberculosis* which escape acute inflammatory cells are engulfed by mononuclear phagocytes and induce delayed type hypersensitivity (Szczepanik *et al*, 2003). Mycobacteria are actually first phagocytosed by alveolar macrophages and conveyed by these cells to draining lymph nodes, and sometimes disseminate through the blood to other parts of the lung and elsewhere in the body. Meanwhile, mononuclear phagocytes act as antigen presenting cells, giving organism-derived peptides to naive CD4 T lymphocytes via class II molecules, and often undergo morphologic transformation into epithelium-like or epithelioid cells (Segovia-Juarez *et al*, 2004). Aggregations of these cells, usually surrounded by lymphocytes, are called granulomas. Epitheliod cells are able to fuse together to form multinucleated giant cells or Langhans giant cells. This lesion is usually evoked by relatively slowly dividing infectious agents such as mycobacteria. Interaction between mycobacteria-activated T-cells and macrophages results in direct toxicity to mycobacteria due to a lack of oxygen and contributes to necrotic caseous tissue damage (Guirado *et al*, 2006).

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Mast cells, inflammatory cells typically found in relatively large numbers in the mucosa and near blood or lymphatic vessels, are likely to be one of the first inflammatory cells to make contact with infectious agents and, following activation, release proinflammatory cytokines, protease enzymes, and inflammatory mediators (Segovia-Juarez *et al*, 2004; Guirado *et al*, 2006). Additionally, mast cell mediators are indispensable for mobilizing and recruiting various other inflammatory cells to the site of infection. *In vitro* study demonstrated that mast cells can directly recognized protein antigens of *M. tuberculosis* and have the potential to play an active role in mediating host response (Muñoz *et al*, 2003). Moreover, mast cells participate in the chronic phase of host defense immunity, including delayed type hypersensitivity. Activated mast cells have to release vasoactive serotonin and TNF-α for endothelial activation and subsequent extravasation of circulating T cells as a first step in order to control infectious agents (McHale *et al*, 1999; Szczepanik *et al*, 2003).

Although previous research demonstrated the cellular response to *M. tuberculosis* was enhanced by the chemical products of mast cells, quantity assessment of mast cells associated with granulomatous formation has never been done. This study investigated the correlation between mast cell numbers and several microscopic components of granulomatous inflammation, which are responsible for infection with *M. tuberculosis*.

MATERIALS AND METHODS

The authors selected 45 cases with granulomatous lymphadenitis between 1999 and 2006 retrieved from the surgical pathology files of King Chulalongkorn Memorial Hospital. All lymph nodes in our study were positive for *M. tuberculosis*, whether by culture or polymerase chain reaction technique.

All four-micrometer-thick hematoxylin and eosin stained slides were reviewed. Components of granulomas, comprised of epithelioid histiocytes, caseous necrosis, and multinucleated giant cells were semiquantitatively stratified (Fig 1). Epithelioid histiocytes and caseous necrosis were scored as 1, 2, 3 or 4 when they were present in less than 25%, 26-50%, 50-75%, or more than 75% of lymph nodes, respectively. Distribution of multinucleated giant cells within the lymph nodes was considered as not seen, less than 1/3, 1/3 to 2/3 and more than 2/3 of areas. For mast cell determination, the sections were stained with 0.1% aqueous toluidine blue. The number of mast cells within or around granulomas were

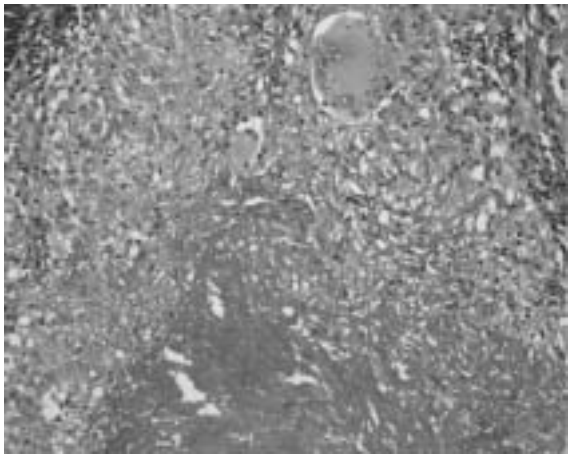

Fig 1–Caseous necrosis, surrounded by crowded palisading epithelioid histiocytes, some of which form multinucleated giant cells.

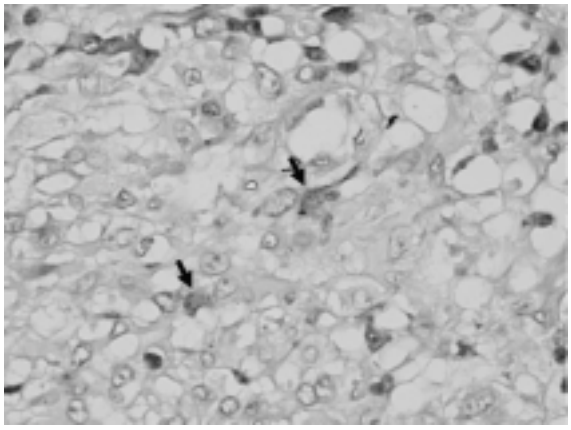

Fig 2–Two mast cells with metachromatic stain (arrow) among scattered epithelioid histiocytes, forming granulomas.

Table 1
Ranges and means of mast cell density among several components of granulomas.

Histological features	No.	Mast cell density (cells/mm^2)		p
		Range	Mean	
Epithelioid histiocyte (score)				
Less than 25% (1)	19	0-12	3	
26-50% (2)	13	0-10	6	
51-75% (3)	10	0-17	6	
More than 75% (4)	3	3-20	10	
Total	45	0-20	5	0.030[a]
Caseous necrosis (score)				
Less than 25% (1)	24	3-20	9	
26-50% (2)	10	0-9	6	
51-75% (3)	7	0-9	6	
More than 75% (4)	4	0-17	4	
Total	45	0-20	5	0.051
MNGC distribution[b]				
Not seen	7	0-12	4	
Equal or less than 1/3	6	0-12	5	
2/3	7	0-10	6	
More than 2/3	25	3-20	12	
Total	45	0-20	5	0.010[a]

[a] = Statistical significance, [b]MNGC = Multinucleated giant cell

identified in the four highest areas and counted using a Nikon Microscope Eclipse E600W, Nikon Digital Camera, and DXM1200F software. The final digitized image was analyzed and represented highest number of mast cells per mm^2 (Fig 2).

Data were analyzed by SPSS for Windows, Version 13. The means of mast cell density among various histological features in different degrees were analyzed, using analysis of variance by two-tailed ANOVA and multiple comparison test. A p-value of <0.05 was taken as the level of significance.

RESULTS

Of the 45 lymph nodes included for analysis, 42 were surgically removed from the neck region and the rest were from the submental, mesenteric, and inguinal regions. The mean age of our subjects was 43 years old. There were 24 males and 21 females.

With reference to Table 1, the mean mast cell density was prone to be increased in lymph nodes replaced by more epithelioid histiocytes, forming a granuloma. Additionally, in lymph nodes with more than 75% (score 4) of epithelioid histiocytic involvement, the mean mast cell density was significantly higher than those with less than 25% (score 1) (p = 0.030). The correlation between mean mast cell density and the distribution of multinucleated giant cells had a similar tend. The more multinucleated giant cells, the more mast cell infiltration. When compared with the other groups, the mean of mast cell density in the lymph nodes with more than 2/3 the area with multinucleated giant cell distribution was higher with a p = 0.010. Although the means of mast cell density had a propensity to be reduced in lymph nodes destroyed by caseous necrosis, this observation was not statistically significant.

DISCUSSION

Tuberculosis is still one of the leading causes of death, particularly in developing countries, including Thailand (Lertmaharit *et al*, 2005). Although granulomatous inflammation was identified long ago as being an important immune response against infection with *M. tuberculosis*, the mechanisms that control their development are little known (Szczepanik *et al*, 2003; Segovia-Juarez *et al*, 2004). To our knowledge, the present study is the first to describe the quantity of mast cells and their relationship to the histopathological alterations responsible for *M. tuberculosis*. We demonstrated that mast cell density has an upward tend in *M. tuberculosis*-infected lymph nodes with more granulomatous involvement. In addition, mast cells even have increasing numbers in nodes with large numbers of multinucleated giant cells.

We observed that the more granulomas involved, the more mast cells. After infection with *M. tuberculosis*, mast cells play a critical role in both acute and chronic phases of the human immune response. In animal models, mast cell numbers and their intracytoplasmic granules were ultrastructurally observed to be increased during the first few days after *M. tuberculosis* infection (Ratnam *et al*, 1977). In addition, mast cells still persist in the chronic state for degranulating substances, eliciting mononuclear cell recruitment to develop granulomas for the purpose of clearing or restricting the growth of microorganisms at the sites of infection (von Stebut *et al*, 2003).

Mast cells are multifunctional, tissue dwelling cells which can release various kinds of mediators (Marshall, 2004). Histamine, a common mast cell product, has the potential of playing an important role in the immune regulatory response of *M. tuberculosis*. This biogenic amine preferentially enhances Th1 responses by triggering histamine type 1 receptor, whereas Th2 responses are negatively regulated (Muñoz *et al*, 2003). The induction of Th1 cells is an indispensable signal because the mechanism of delayed type hypersensitivity relied mainly on Th1-secreted cytokines (Kataoka *et al*, 2005; Mann-Chandler *et al*, 2005). TNF-α released by mast cells is considered a crucial mediator of both protection and pathology during tuberculosis. Even though de novo synthesis of TNF-α occurs in mast cells, it is conceivable that some of the TNF-α detected in the extracellular medium is prestored in mast cell granules (Muñoz *et al*, 2003). It is noteworthy that mast cells are the only cell type in the body that can store this cytokine in its preformed state before releasing after cell activation. With early infection, mast cell TNF-α may be critical for the activation of macrophages and immature monocytes to form granulomas (Muñoz *et al*, 2003; Szczepanik *et al*, 2003). During the more chronic states of infection, TNF-α appears especially important for maintaining the integrity of the granulomas and for the control of bacteria, because its depletion results in desegregation of the granulomas and exacerbation of the infection (Mizuno *et al*, 2001). IL-6, another proinflammatory cytokine, is produced by mast cells after activation by *M. tuberculosis*. Experimental animal models of tuberculosis have shown that it plays a role at the beginning of the infection and during the early phases of granuloma formation (Law *et al*, 1996; Muñoz *et al*, 2003).

We demonstrated that the population of mast cells tended to increase in granulomatous lesions with more multinucleated giant cells. Multinucleated giant cells or Langhans giant cells are enhanced by fusion of monocytes or macrophages after direct contact with mycobacteria in combination with cytokine-containing supernatants and membrane-bound molecules of mycobacteria (Gasser and Most, 1999). The fusion rate was also distinctively increased when they were induced by TNF-α, and anti-TNF-α rabbit antibody can suppress the fusion process (Takashima *et al*, 1993). As aforementioned, TNF-α which is released by mast cells not only enhances cell fusion, but also participates in several steps

of granuloma formation. This *in vitro* study disclosed a high rate of cell fusion in freshy monocytes and the rate gradually decreased with time of the culture. Because the ability to kill intracelluler microbacteria is reduced in macrophages, fusion with monocytes just reaching the site of inflammation under mast cell influence may represent an attempt to restore this capacity (Most *et al*, 1997).

In conclusion, in tuberculous lymphadenitis, we believed that increasing mast cell density may be an important part of pathogenesis for expressing granulomas as well as multinucleated giant cell formation in order to control and eradicate *M. tuberculosis*.

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